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RESEARCH ARTICLE OPEN ACCESS

Oral Administration of Ethanol Extract of *Myristica fragrans* Elicited Hyperactivity, Anxiety and Reduced Dopamine Level in Rats

Ethanol seed extract of myristica fragrans and Anxiety

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Abstract
Objective: *Myristica fragrans*, a common kitchen spice, is reported to have psychoactive properties, producing hallucinations and fear. The psychoactive action is attributed to the presence of myristicin. This study aimed to assess the effects of ethanol seed extract of *Myristica fragrans* (ESMF) on anxiety-like behaviour and dopamine levels in rats.
Materials and Methods: Thirty-six male Wistar rats were divided into six groups of six rats each. Groups I (control, received 2 ml/kg distilled water); Group II (received 2 ml/kg of tween 80 Oil); Groups III, IV and V received ESMF (500 mg/kg; 1000 mg/kg and 1500 mg/kg, respectively) and group VI received methylphenidate (1 mg/kg) as standard stimulant. All administration was done orally for 28 days. The rats were subjected to neurobehavioral tests: an open field test to assess hyperactivity and an elevated plus maze to assess anxiety-like behaviour. Brain samples were harvested and homogenized for neurochemical analysis of dopamine using a dopamine kit.
Results: Hyperactivity was observed as increased line crossing and total distance travelled in the ESMF (500mg/kg)-treated group when compared with the control group and reduced anxiety-like behaviour by reduced frequent close arm entry and duration. Dopamine levels were reduced in the 1500 mg/kg ESMF-treated group compared to the control.
Conclusion: Exposure to ethanol seed extract of *Myristica fragrans* elicited anxiety-like behaviour. ESMF at a dose of 500mg/kg possesses anxiolytic properties, while at a dose of 1500 mg/kg; elicits anxiety by a reduction in dopamine levels in rats.

Keywords: Nutmeg, Alterations, Myristicin, Anxiety, Stimulant

Plain English Summary

Oral administration of ethanol seed extract of Myristica fragrans in male rats reduces fear at low doseS. On the contrary, it causes fear at high doseS by reducing brain dopamine level.

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Introduction

Myristica fragrans is commonly known as Nutmeg. It is a food-spicing agent in most kitchens, it gives a pleasant smell or aroma that excites the psychomotor area (medial prefrontal cortex, basal ganglia, thalamus and cerebellum) of the brain responsible for increased appetite for food (1, 2). Nutmeg has been reported to have psychoactive properties of producing anxiety, hallucination and fear (3), and it is often used as a stimulant by prisoners and seamen (4), it is used in cake making, and as spices in the kitchen and can lead to more exposure to it which could result to its abuse and subsequent addiction. Its psychoactive action is due to the presence of Myristicin and elemicin which have been reported to have anti-depressant action (5, 6). Addictive Substances have been reported to increase dopamine levels in the brain (7), on the contrary, long-term exposure to addictive substances can lead to a decrease in dopamine levels (8).

Dopaminergic neurons arising from the compacta part of the substantia nigra, send their projections to the striatum (9, 10), in that way, they are involved in motor coordination, associative learning, and reward recognition. Degeneration of the dopaminergic neurons has been implicated in the aetiology of movement disorders, hallucination, and anxiety (11).

Anxiety is both a physiological and a psychological condition that is made up of cognitive, somatic emotional, and behavioural components, that act together to create feelings of anger, fear, apprehension, and worry typical of anxiety (12). Anxiety is a feeling of apprehension due to anticipated danger (13). All forms of anxiety can stimulate and activate the stress response that will result in to release of neurotransmitters of the sympathetic nervous system, the catecholamines of which Dopamine is one (14).

There have been reports on the neurotoxic effects of nutmeg consumption on several areas of the brain (4), yet the mechanisms of its neurotoxicity are yet to be completely unravelled (15). With the increase in nutmeg usage as a spice in food and baking and its prolonged exposure as a substance to make one “high” (4), it’s imperative to assess its impact on behaviour such as anxiety. Consequently, this research attempted to assess the effect of ethanol seed extract of *Myristica fragrans* (nutmeg) on anxiety-like behaviour, and dopamine levels in Wistar rats.

Materials and Methods

Animals

Thirty-six (36) male Wistar rats weighing 130-150 grams were obtained and housed in the animal breeding house of the Department of Human Anatomy, Ahmadu Bello University (ABU), Zaria, Nigeria. The Rats were divided into six groups of six rats each. Groups I (control, received 2 ml/kg distilled water); Group II (received 2 ml/kg of Tween 80 Oil); Groups III, IV, and V received ESMF (500 mg/kg extract; 1000 mg/kg extract and 1500 mg/kg extract, respectively) and group VI received 1 mg/kg of methylphenidate as standard stimulant orally (16). Rats were subjected to an open field test to assess locomotor activity, and an elevated plus maze to assess anxiety-like behaviour, and by the end of administration (day 28), the rats were euthanized after which the skulls were dissected through a mid-sagittal incision and brains harvested. The harvested brains were homogenized in phosphate-buffered saline (PBS, 0.1M, pH 7.4) for subsequent neurochemical analyses.

Purchase of Powdered nutmeg and Methylphenidate

Nutmeg powder manufactured by Tiger Foods Limited Nigeria, was obtained from a reputable supermarket in Kaduna. Methylphenidate manufactured by Novartis Pharmaceuticals Corporation New Jersey USA was obtained from a reputable Pharmaceutical Store in Kaduna and used as a standard (reference) central nervous system stimulant drug.

Extraction Procedure

Ethanol extraction of *Myristica fragrans* was conducted using the maceration method as described by (17). Ethanol was used as the solvent of extraction for maximum yield of the phytochemical of interest (secondary metabolites: alkaloid; myristicin). The extraction was conducted in the Department of Chemistry, Faculty of Natural Sciences, Kogi State University, Anyigba. Briefly, the protocol of extraction is as follows: Powdered *Myristica fragrans* seed weighing 750 grams was soaked in 2.550 litres of absolute ethanol and allowed to stay for 72 hours for maximum yield. The mixture was then filtered using a vacuum pump and evaporated to dryness in a steam bath at 40-50^{o}C.

Percentage yield
The percentage yield of the extract was determined by weighing the powdered *Myristica fragrans* before extraction and after extraction and computed using the formula (18):

$$\text{Percentage yield} = \frac{weight\ of\ extract}{weight\ of\ powdered\ nutmeg} \times 100$$

Neurobehavioral assessments
The following neurobehavioral assessments were conducted: an open field test for locomotor activity and an Elevated Plus Maze test for anxiety-like behaviour. All assessments were conducted in the Neuroanatomy and Neuroscience Laboratory of the Department of Human Anatomy, ABU, Zaria.

Open Field Test
The open-field experiment was carried out to assess hyperactivity and motor activity as described by Adebiyi (19). The open-field apparatus was constructed with the following dimensions: 100 cm × 100 cm ×40cm, and the centre zone is a square 18cm away from the wall of the open field. Each rat was placed at the centre of the open field apparatus and allowed to explore for 5 minutes. The open field test was conducted before the commencement of administration of the extract and at the end of week four (day 28). The following parameters were recorded: Line Crossing and Total Distance Travelled using a staged digital camera. The recorded parameters were computed using a computer running image/ video analysis software (Image J, NIH, US). The Image J Animal Tracker Plug-in tool was used following certain calibrations according to the manufacturer's instructions.

Elevated plus maze
An elevated plus maze (EPM) test was conducted to assess anxiety-like behaviour. The protocol described by Walf was employed (20). EPM apparatus with the dimensions: 50 cm × 10 cm × 20 cm, a central square of 10 cm × 10 cm and 50 cm height from the floor was used. Each rat was placed in the central area of the maze facing one of the open arms and allowed to explore for 5 minutes. Parameters such as rearing, grooming, the number of entries into the close and open arm and the time spent in each arm were recorded. The maze was cleaned with a solution of methylated spirit and allowed to dry between tests to remove any odour residue from the previous rat. The test was conducted before administration of the extract (Pre-test), at the end of week one (Test 1), at the end of week two (Test 2) and at the end of week three (Test 3).

Dopamine Assessment
The tissue homogenate was used to assess dopamine levels using ELISA kits (ER 1728), Wuhan China. The tissue homogenates were collected in pre-rinsed plastic tubes. Tubes were then centrifuged at 1000Xg for 10 minutes and kept at -20^{o}C. Brain dopamine level was then determined by the ELISA method according to the manufacturer's instructions.

Data Analysis
The data obtained were analyzed using Graph Pad Prism version 8, and results were expressed as mean ± standard error of the mean (SEM). Two-way repeated measure ANOVA followed by a *Tukey post hoc* test was performed and $P \leq 0.05$ was considered to be statistically significant.

Results
The Rats' performance on the elevated plus maze showed a significant increase in Open-arm and Closed-arm frequencies in control and 500 mg/kg extract (EX) groups when pre-test and test 1 were compared to test 3, $p<0.05$. as indicated in figure Ia and Figure Ib respectively. Also, a significant increase was observed when 500 mg/kg EX pre-test and test 1 were compared to test 3 in open-arm duration and closed-arm duration as shown in Figures 1c and 1d respectively. Rats treated with 500 mg/kg of the extract spent more time in both the open arm and closed arm of the maze and also tended to explore both arms more compared to rats in other groups that received higher doses of the extracts. Rearing and grooming behaviour of rats on elevated plus maze test revealed a significant decrease of $p<0.05$ when control and 500 mg/kg EX pre-test and test 1 were compared with test -2 as indicated in Figures 1e and 1f, respectively. In Table 1, rats in the 500 mg/kg treated group showed a significant ($p<0.05$) increased number of lines crossed and total distance travelled in the open field test compared to the control. A significant ($p<0.05$) decrease in line crossing and distance travelled was observed in the groups that were treated with 1000 mg/kg and 1500 mg/kg of the extract as shown in Table 1. Figure 2 revealed

a significant decrease in dopamine levels in the 1500 mg/kg treated group compared to the control.

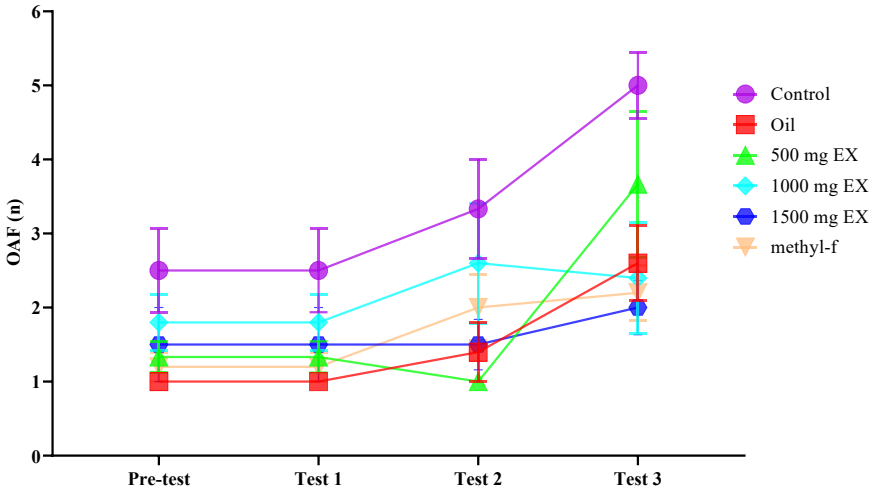

Figure 1a: Effect of ESMF on elevated plus maze (open arm frequency, OAF) performance in rats
n= 6; mean ± SEM, Two-ways Repeated measures ANOVA, *Tukey post-hoc* test, significant difference when control and 500 mg/kg EX pre-test and test 1 were compared to test 3. **EX**: Ethanol seed extract of *Myristica fragrans* (ESMF)*;* **methyl-f**: Methylphenidate (standard drug). pre-test (before treatment). Test-1 (week 1), Test-2 (week 2), Test-3 (week 3).

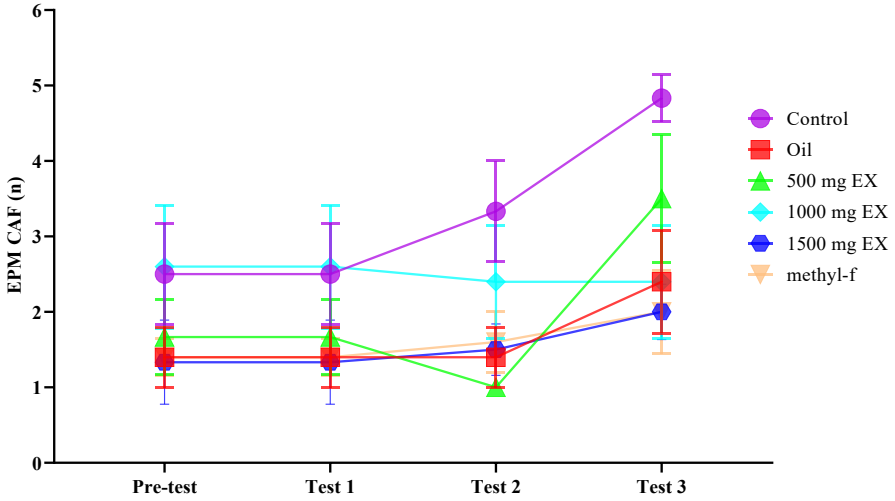

Figure 1b: Effect of ESMF on elevated plus maze (closed arm frequency, CAF) performance in rats
n=6; mean ± SEM, Two-ways Repeated measures ANOVA, *Tukey post-hoc* test, significant difference when control and 500 mg/kg EX pre-test and test 1 were compared to test 3. **EX**: Ethanol seed extract of *Myristica fragrans* (ESMF)*;* **methyl-f**: Methylphenidate (standard drug). Test-1 (week 1), Test-2 (week 2), Test-3 (week 3).

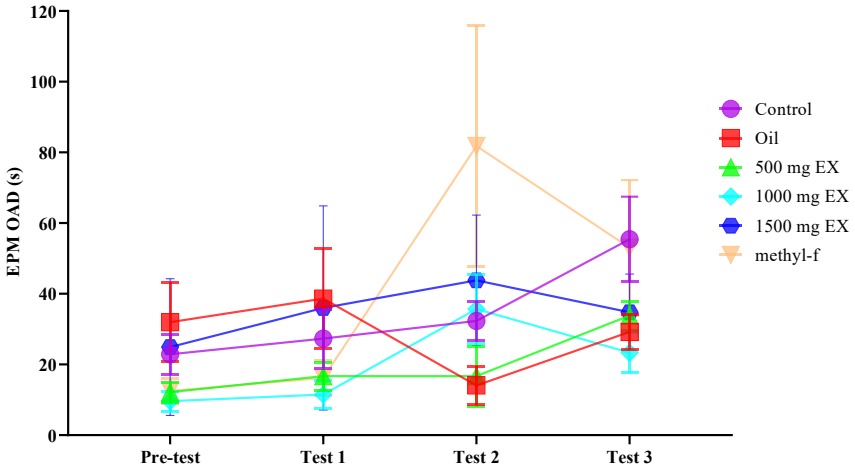

Figure 1c: Effect of ESMF on elevated plus maze (open arm duration, OAD) performance in rats
n=6; mean ± SEM, two-way repeated measures ANOVA, *Tukey post-hoc* test, significant difference when 500 mg EX pre-test was compared to test 3. **EX**: Ethanol seed extract of *Myristica fragrans* (ESMF)*;* **methyl-f**: Methylphenidate (standard drug). Test-1 (week 1), Test-2 (week 2), Test-3 (week 3).

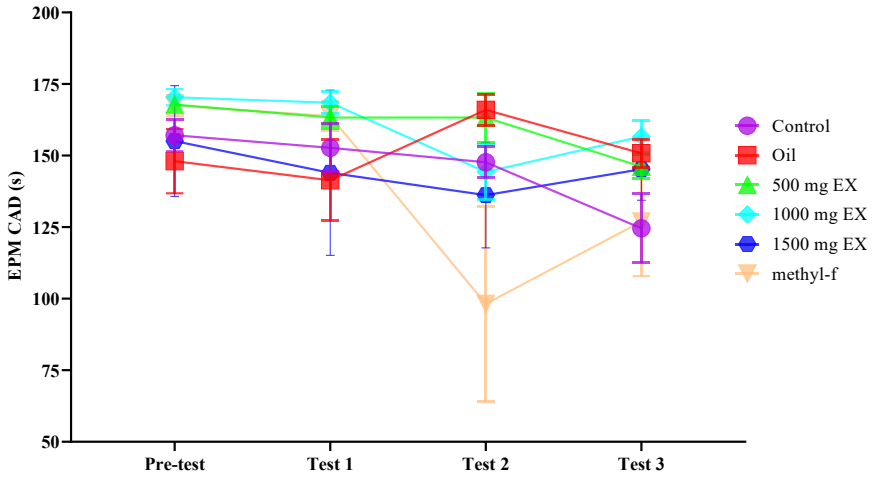

Figure 1d: Effect of ESMF on elevated plus maze (closed arm duration, CAD) performance in rats
n=6; mean ± SEM, two-way repeated measures ANOVA, *Tukey post-hoc* test, significant difference when 500 mg EX pre-test was compared to test 3. **EX**: Ethanol seed extract of *Myristica fragrans* (ESMF)*;* **methyl-f**: Methylphenidate (standard drug). Test-1 (week 1), Test-2 (week 2), Test-3 (week 3).

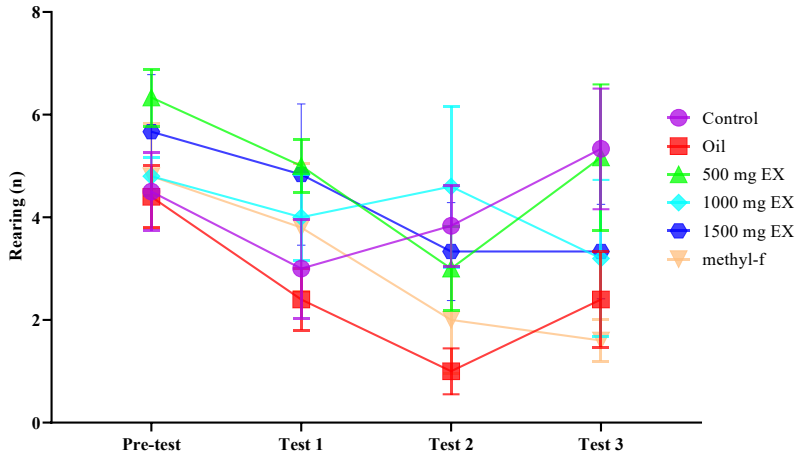

Figure 1e: Effect of ESMF on elevated plus maze (rearing) performance in rats

n=6; mean ± SEM, Two-ways Repeated measures ANOVA, *Tukey posthoc* test, a significant decrease when control, oil, and 500 mg EX pre-test were compared to test-1 and test-2; **EX**: Ethanol seed extract of *Myristica fragrans* (ESMF)*;* **methyl-f**: Methylphenidate (standard drug). Test-1 (week 1), Test-2 (week 2), Test-3 (week 3).

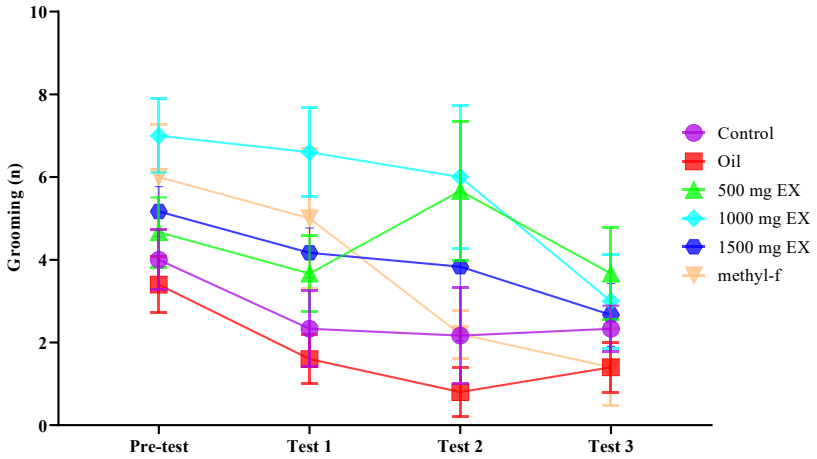

Figure 1f: Effect of ESMF on elevated plus maze (grooming) performance in rats

n= 6; mean ± SEM, Two-ways Repeated measures ANOVA, *Tukey post-hoc* test, significant decrease when oil pre-test, was compared to test 1 and test 2 when 500 mg/kg pre-test was compared to test 1. EX; Ethanol seed extract of *Myristica fragrans* (ESMF)*;* **methyl-f**: Methylphenidate (standard drug). Test-1 (week 1), Test-2 (week 2), Test-3 (week 3).

Table 1: Mean Open Field Performance of Wistar rats Treated with ESMF

Groups	Line crossing (cm)	Distance covered (cm)
Control	6.5±0.43	816.25±152.45
Oil	16.20±0.49^{a}	1532.38±293.85
500 mg/kg Ex	11.17±1.17^{ab}	1951.45±149.94^{a}
1000 mg/kg Ex	7.8±0.37^{bc}	1406.31±325.32
1500 mg/kg Ex	6.83±0.4^{bc}	991.23±105.11^{ac}
1mg/kg methyl-f	5.2±0.37^{bc}	890.66±108.78^{ac}

n = 6, mean ± SEM, One-way ANOVA. *Turkey post hoc test,* a = p<0.05 when compared to the control group, b = p<0.05 when compared to the Oil group, and c = p<0.05 when compared to the 500 mg/kg Extract group. Methyl-f: methylphenidate (standard drug)

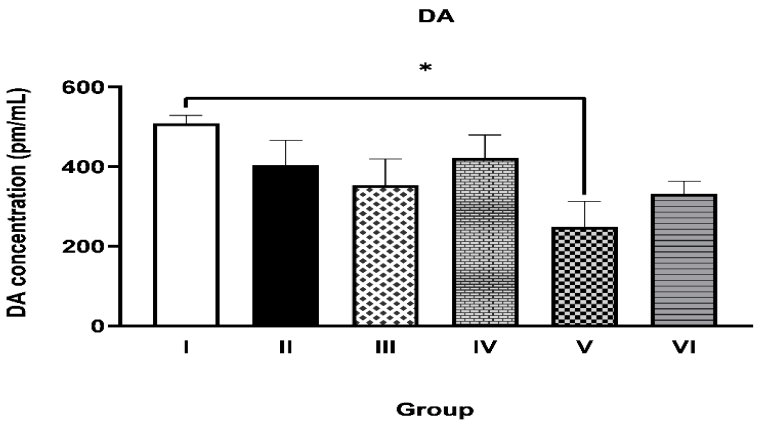

Figure 2: Mean DA level of Wistar rats Treated with ESMF.

n=6; mean± SEM; One way ANOVA; *Tukey post hoc* test showed significant decrease ($p < 0.05$); Group I: Distilled water (2 ml/kg); Group II: Olive oil (2ml/kg); Group III: ESMF (500 mg/kg); Group IV: ESMF (1000 mg/kg); Group V: ESMF (1500 mg/kg); Group VI: Methyphenidate (1mg/kg); ESMF: Ethanol seed extract of Myristica fragrans; DA: Dopamine.

Discussion

To assess the effect of *Myristica fragrans* on anxiety-like behaviour, Rats were subjected to an Elevated Plus Maze (EPM) and Open field test (OFT) respectively.

Anxiety-like behaviour was observed as reduced open arm frequency and duration, reduced line crossing and total distance travelled in the 1500 mg/kg treated group. On the contrary, hyperactivity was observed as increased line crossing and total distance travelled in the 500 mg/kg treated group. Rearing and grooming showed a remarkable decrease when the group administered 500mg/kg was compared to the control. This is due to the anti-depressant and anxiolytic actions of *Myristica fragrans* at a low dose (21). The findings here indicate that ethanol seed extract of *Myristica fragrans* (ESMF) elicited anxiolytic behaviour at 500 mg/kg dose, but anxiogenic behaviour at ESMF 1500 mg/kg dose in rats. The number of lines crossed and distance travelled in the open field test is an indication of hyperactivity, anxiety, and locomotor activity. The results of the open field test showed remarkable hyperactivity and locomotor activity in the 500 mg/kg treated group as indicated by the number of line crossings and distance travelled. A general decrease in locomotor activity and the number of lines crossed observed in the group that was treated with 1500 mg/kg doses of the extract indicated anxiogenic behaviour. These findings are in agreement with the work of Sonavane (21), who reported that the anxiolytic actions of nutmeg are only elicited at a lower dose and that at a higher dose, *Myristica fragrans* decreases locomotor activity in mice.

The decreased locomotor activity can be attributed to a decrease in dopaminergic transmission or dopamine level. This can be attributed to the toxic effect of the extract because of exposure to its high dose and can be corroborated with the reduced dopamine level observed in the group that was administered 1500 mg/kg of the extract.

These results are in agreement with the work of Sonavane, *et al* (21), who reported that ethanol seed extract of *Myristica fragrans* has a complex CNS activity, it is anxiogenic and reduces locomotor activity in Rats at high doses and anxiolytic at low dose.

The results from the assessment of Dopamine (DA) level in tissue revealed a remarkable decrease in dopamine level in the group administered 1500mg/kg of extract compared to control. The result agrees with the work of Sabbar *et al*., (22), and Seddik *et al*. (23), who reported that neurotoxic substances generally induce a reduction in the catecholaminergic transmission either by inhibiting the synthesis of DA and its release or by inhibiting their postsynaptic receptors D2 (24). Reduction in DA could interfere with the nigrostriatal dopaminergic path which could result in a reduction in the dopaminergic transmission of the nigrostriatal system (25). The decreased DA activity observed in this study could be attributed to the neurotoxic effect of the ethanol seed extract of *myristica fragrans* at the 1500 mg/kg dose.

Conclusion

In conclusion, exposure to ethanol seed extract of *Myristica fragrans* resulted in hyperactivity and anxiolytic effect at 500 mg/kg dose, anxiogenic

effect at 1500 mg/kg dose, and reduced dopamine level at 1500 mg/kg dose in the brain of rats.

List of Abbreviations

ESMF: Ethanol Seed extract Myristica Fragrans
EX: Extract (Ethanol seed extract Myristica fragrans)
EPM: Elevated Plus Maze
OFT: Open Field Test
DA: Dopamine
CNS: Central Nervous System
CAF: Closed Arm Frequency
CAD: Closed Arm Duration
OAF: Open Arm Frequency
OAD: Open Arm Duration

Declarations

Ethics considerations and consent to participate

Ethical approval was obtained, and all experimentation and animal handling were according to the Ahmadu Bello University Ethical Committee on Animal Use and Care guidelines. (ABUCAUC/2023/053).

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license. We otherwise convey all copyright ownership, including all rights incidental thereto, exclusively to the journal when published.

Availability of data and materials

The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

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Author Contributions

AS: conceptual design, data analysis, laboratory work, acquisition of funding, drafting of manuscript. AAN: data curation, data analysis, result interpretation, supervision, laboratory work. MSA: supervision, final editing and approval of manuscript, laboratory work, and supervision. OSB: supervision, final approval of the manuscript, data analysis and interpretation. MIJ: laboratory work, data interpretation, and drafting of the manuscript. EO: laboratory work, proofreading of the final manuscript, editing of manuscript. OOD: supervision, proofreading and final approval of manuscript.

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